



Platelet Rich Plasma Versus Minoxidil on Hair Growth in Male Albino Rat Model of Androgenic Alopecia. Histological and Immunohistochemical study

Thesis

*Submitted for the Partial Fulfillment of Master Degree
in Histology and Cell Biology*

Submitted by

Nancy Sultan Saad Sultan

M.B, B.Ch, Faculty of Medicine - Ain Shams University

Under Supervision of:

Prof. Dr. Hoda Fouad Nada

*Professor of Histology and Cell Biology
Faculty of Medicine- Ain Shams University*

Ass. Prof. Dr. Sara Abdel Gawad Elsebay

*Assistant Professor of Histology and Cell Biology
Faculty of Medicine- Ain Shams University*

Ass. Prof. Dr. Dalia Alaa El-Din Aly El-Waseef

*Assistant Professor of Histology and Cell Biology
Faculty of Medicine- Ain Shams University*

Faculty of Medicine
Ain Shams University

2020

Acknowledgment

First and foremost, thanks to God the kindest and the most merciful to whom I relate any success in achieving any work in my life.

*There are no words by which I can express my sincere thanks and deepest gratitude to **Prof. Dr. Hoda Fouad Nada**, Professor of Histology, Faculty of Medicine, Ain Shams University, for her consistent support and encouragement. Her wide experience, precious instructions and supervision helped me achieve this work. It was such a great honor to work under her guidance.*

*I'm deeply indebted and grateful to **Ass. Prof. Dr. Sara Abdel Gawad Elsebay**, Assistant Professor of Histology, Faculty of Medicine, Ain Shams University, for her valuable advice, great help, and sincere efforts that served much in the construction of this work. She has always been helpful in a way that made the completion of this work much easier. It was a pleasure to proceed with this work under her supervision.*

*I find no words by which I can express my sincere and deep thanks to **Ass. Prof. Dr. Dalia Alaa El-Din Aly El-Waseef**, Assistant Professor of Histology, Faculty of Medicine, Ain Shams University, who supplied me with valuable advice, meticulous observation and precious instructions to finish this work in that form.*

I would like to offer special thanks to all my professors and my colleagues in Histology Department, Faculty of Medicine, Ain Shams University, for their warm kindness, valuable advice, and continuous support.

Finally, I found no words to express my thanks, gratefulness, respect, and love to my parents, husband, and family for their care and support to achieve my success.

Nancy Sultan Saad

List of Contents

Title	Page No.
List of Tables	i
List of Diagrams	ii
List of Histograms	iii
List of Abbreviations.....	iv
Abstract	v
Introduction	1
Aim of the Work	9
Review of Literature	10
Materials and methods	34
Results	46
Discussion	137
Summary	150
Conclusion and Recommendations.....	154
References	155
Arabic summary.....	----

List of Tables

Table No.	Title	Page No.
Table (1):	Changes in epidermal thickness expressed in μm represented by mean \pm standard deviation in the different experimental groups:	125
Table (2):	Changes in area percentage of collagen represented by mean \pm standard deviation in the different experimental groups:	127
Table (3):	Showing changes in total number of hair follicles represented by mean \pm standard deviation in the different experimental groups	129
Table (4):	Showing changes in number of anagen hair follicles represented by mean \pm standard deviation in the different experimental groups	131
Table (5):	Changes in number of telogen hair follicles represented by mean \pm standard deviation in the different experimental groups	133
Table (6):	Showing changes in number of cells showing positive immune reaction for Ki 67 immunostaining represented by mean \pm standard deviation in the different experimental groups.....	135

List of Diagrams

Diag. No.	Title	Page No.
Diagram (1):	The four compartments of the hair follicle	12
Diagram (2):	The hair growth cycle has 3 major phases.....	15

List of Histograms

Histog. No.	Title	Page No.
Histogram (1):	Showing epidermal thickness in the different experimental groups:.....	126
Histogram (2):	Showing area percentage of collagen in the different experimental groups:.....	128
Histogram (3):	Showing total number of hair follicles in the different experimental groups:.....	130
Histogram (4):	Showing number of anagen hair follicles in the different experimental groups:.....	132
Histogram (5):	Showing number of telogen hair follicles in the different experimental groups.....	134
Histogram (6):	Showing number of Ki 67 positive basal epidermal cells in the different experimental groups	136

List of Abbreviations

Abb.	Full term
AGA	<i>Androgenic alopecia</i>
DHT.....	<i>Dihydrotestosterone</i>
EGF	<i>Epidermal growth factor</i>
EM	<i>Electron microscope</i>
ES cells.....	<i>Epidermal stem cell</i>
FGF.....	<i>Fibroblast growth factor</i>
GFs	<i>Growth factors</i>
HGF	<i>Hepatocyte growth factor</i>
IGF.....	<i>Insulin growth factor</i>
KAPs.....	<i>Keratin associated proteins</i>
PBS.....	<i>Phosphate buffer saline</i>
PDGF.....	<i>Platelet-derived growth factor</i>
PRP.....	<i>Platelet rich plasma</i>
SC	<i>Subcutaneous</i>
TGF- β	<i>Transforming growth factor-β</i>
VEGF.....	<i>Vascular endothelial growth factor</i>

Abstract

Background: Androgenic alopecia (AGA) is the commonest cause of hair loss in men with limited treatment options. Platelet-rich plasma (PRP) is defined as an autologous concentration of plasma with a greater count of platelets than that of whole blood. Its action depends on the released growth factors from platelets. It has been investigated and used in numerous fields of medicine. Recently, PRP has received growing attention as a potential therapeutic tool for hair loss.

Aim of the work: This study aimed at comparing the efficacy of PRP therapy with minoxidil therapy in experimentally induced AGA in adult male albino rats.

Materials and Methods: Forty-five male albino rats were divided equally into 5 groups - Group I (the control group). Rats of the remaining 4 groups received subcutaneous (SC) injections of 0.1 ml testosterone for induction of alopecia, after 10 days alopecia was observed. Group II (testosterone group); each rat received subcutaneous injection of 0.1 ml testosterone daily in the marked area for the following 21 days. Group III (minoxidil group); each rat received topical minoxidil daily on the marked area together with SC injection of 0.1 ml testosterone daily in the marked area for the following 21 days. Group IV (PRP); each rat received SC injection of 0.1 ml PRP every 3 days in the marked area, together with daily SC injection of 0.1 ml testosterone in the marked area for the following 21 days. Group V (PRP+ minoxidil group); each rat received SC injection of 0.1 ml PRP every 3 days in the marked area+ topical minoxidil daily on the marked area, together with SC injection of 0.1 ml testosterone daily for the following 21 days. Rats were sacrificed after 31 days from the start of the experiment. Skin samples of size 1cm³x1cm³ were collected from the site of injection and prepared for histological, immunohistochemical, and electron microscopic examination. Morphometrical and statistical analysis were performed.

Results: Testosterone group when compared to the control group showed a significant decrease in mean epidermal thickness, decreased mean number of anagen hair follicles, and increase in mean number of telogen hair follicles, all were statistically significant (P<0.05). Groups III, IV, and V showed a significant increase in mean epidermal thickness, increase in mean number of total hair follicles, increase in mean number of anagen hair follicles (p<0.05), and a decrease in mean number of telogen hair

follicles ($p < 0.05$). All were also statistically significant ($p < 0.05$). Group V showed the best results.

Examination of Mallory stained sections of testosterone group showed perifollicular fibrosis and follicular streamers. Minoxidil group showed less perifollicular fibrosis and less follicular streamers. PRP group and group V also showed less perifollicular fibrosis and streamers, as compared to the previous groups.

Examination of anti-Ki 67 immunohistochemically stained sections in testosterone group showed significant decrease of Ki 67 positive basal epidermal cells, outer root sheath Ki 67 positive stem cells, bulge stem cells and matrix cells, all were statistically significant ($p < 0.05$) compared to control group. PRP and minoxidil groups showed a significant increase in number of Ki 67 positive cells ($p < 0.05$) as compared to the control group and the testosterone group. Group V showed numerous Ki 67+ cells which was statistically higher than the other experimental groups.

When comparing the PRP group and the minoxidil group, PRP group showed significantly better results than the minoxidil group in all the above-mentioned parameters. However, the best results were obtained when simultaneously applying both PRP and minoxidil.

Conclusion: Our data suggest that PRP injections have a better therapeutic effect than minoxidil, and that both PRP and minoxidil when administered together have the best effect on experimentally induced androgenic alopecia in adult male albino rats.

Keywords: Platelet Rich Plasma versus Minoxidil, Hair Growth, Male Albino Rat Model, Androgenic Alopecia

INTRODUCTION

Hair loss refers to loss of some or all hairs on the head or the body which is caused by a wide range of factors from environmental to genetics. Androgenic alopecia is the most common cause of hair loss affecting men and women at different ages (*Gkini et al., 2014*).

In human, the hair cycle is divided into 3 stages: Anagen (the growing phase, 90%): lasts for 2-7 years and it determines the length of our hair, Catagen (regression phase): It's a transitional stage that lasts about 10 days, the hair follicle shrinks and detaches from hair papilla, Telogen (resting phase): lasts for about 3 months. Whilst the old hair is resting in telogen phase, a new hair begins the growth phase, this is followed by shedding of the hair (exogen) and growth of the new one (*Geyfman et al., 2014*).

Hair loss is a symptom of disruption of the hair cycle, usually a consequence of premature entry into catagen or an extended telogen phase (*Baker, 2012*). The most common type of hair loss is androgenic alopecia (AGA). The term androgenic alopecia is frequently used to express the patterned loss of scalp hair in genetically vulnerable men and women (male pattern hair loss and female pattern hair loss). AGA is a polygenetic condition with a wide spectrum of severity, age of onset, and scalp location of hair loss. In men, hair loss frequently occurs in a characteristic “horseshoe” pattern, in which hair is lost on

the front and top of the head but not on the back and sides. Although AGA is as frequent in women as it is in men, the hair loss is usually less severe, doesn't occur in the characteristic pattern, and is manifested as an overall thinning (*Vary, 2015*). AGA is believed to be due to androgens which lead to shortening of the anagen phase, increasing number of hair follicles in catagen and telogen phase, and delaying telogen-to-anagen transition. Moreover, the follicular miniaturization by androgens results in transformation of terminal follicles to vellus-like follicles, which produces thinner and shorter hairs (*Orasan et al., 2016*)

Minoxidil stimulates hair growth by increasing cutaneous blood flow, and it is also a potassium channel opener causing hyperpolarization of cell membranes, thus it allows more oxygen, blood, and nutrients to the follicles (*Roberts et al., 2014; Goren et al., 2015*).

Recently, platelet-rich plasma has been demonstrated in the treatment of alopecia. The activation of platelet alpha granules releases numerous growth factors, including transforming growth factor (TGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and interleukin-1. It is proposed that growth factors released from platelets may act on stem cells in the bulge area of the follicles, stimulating the development of new follicles and promoting neovascularization (*Cervantes et al., 2018*).

AIM OF THE WORK

This study was performed to compare the efficacy of minoxidil and platelet rich plasma on experimentally induced androgenic alopecia in adult male albino rats.

REVIEW OF LITERATURE

Histological structure of the hair:

Hairs are elongated keratinized structures that form within epidermal invaginations, the hair follicles. The colour, size, shape and texture of hairs vary according to age, genetic background, and region of the body.

All skin has at least minimal hair except the glabrous skin of the palms, soles, lips, glans penis, clitoris, and labia minora

Hair shaft is the part projecting above the surface of the skin. Hair root is the part embedded under the skin surface and is surrounded by the hair follicle (*Ross and Pawlina, 2016*).

Hair shaft: (*Ross and Pawlina, 2016*)

Hair shaft consists of 3 layers: cuticle, cortex, and in some cases medulla

- a) **Cuticle:** It is the outermost layer of the hair shaft. It consists of several layers of overlapping semitransparent keratinized squamous cells. These cells resemble fish scales or roof tiles with their free edges lying away from the hair follicle. The cuticle protects the hair from physical and chemical damage and determines its porosity.

- b) **Cortex:** It is the middle layer of most thick hairs and the innermost layer of some thinner finer hairs where the medulla is absent. It is the largest layer and accounts for 80% of total hair mass. It is composed of cortical cells filled with hard keratin intermediate filaments. Each filament is surrounded by an amorphous space containing keratin associated proteins (KAPs). These high sulfur KAPs are responsible for rigidity of the hair shaft by causing extensive cross-linking between keratin intermediate filaments by disulfide bonds. The cortex determines texture, elasticity, and colour of the hair.
- c) **Medulla:** The medulla forms the central part of the shaft and contains a column of large, loosely connected thin transparent moderately keratinized vacuolated cells containing soft keratin, and air spaces. The medulla is present only in thick hairs

Hair follicle:

The hair follicle is a dynamic organ that resides in the dermal layer of mammalian skin. It is responsible for the production and growth of hair through a complex interaction between hormones, neuropeptides, and immune cells.

The hair follicle begins at the surface of the dermis. For follicles that produce terminal hairs, the hair follicle extends into the deep dermis, and sometimes even subcutis, while

follicles that produce vellus hairs extend only to the upper reticular dermis

The hair follicle develops as a downgrowth of the epidermal cells into the dermis. It is divided into 2 distinct parts: upper part consisting of infundibulum and isthmus, whereas the lower part comprises the bulbar and suprabulbar regions. The upper follicle remains constant, while the lower part has continuous cycles of regeneration

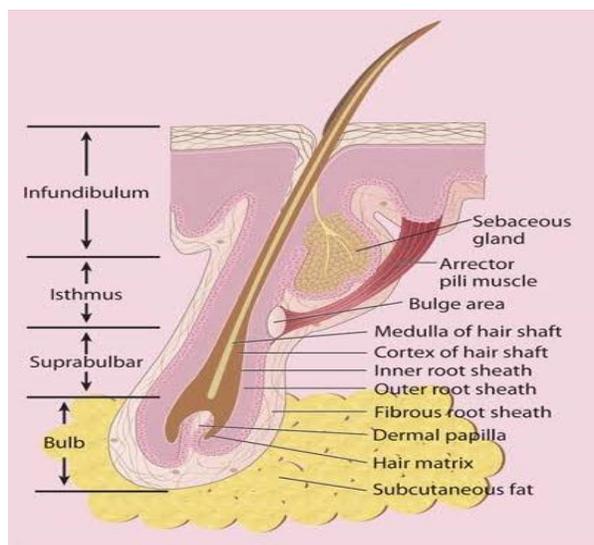


Diagram (1): The four compartments of the hair follicle
(*Goldsmith et al., 2008*).

The infundibulum is the upper portion of the follicle which extends from the surface of the epidermis and extends to the opening of the sebaceous duct. It is part of the pilosebaceous canal which is used as a route for discharge of sebum